Chapter 5
Intrauterine effects of maternal smoking on sensitization, asthma and COPD

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CHAPTER 5

Intrauterine Effects of Maternal Smoking on Sensitization, Asthma, and Chronic Obstructive Pulmonary Disease

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One third of women continue to smoke during early pregnancy, although evidence for detrimental effects of *in utero* smoke exposure on fetal growth and development are rising. A number of epidemiologic studies have shown that prenatal exposure to environmental smoke is an independent risk factor for poor lung function, wheezing, and the development of (possibly nonatopic) asthma. Epidemiologic data on the effect on development of allergic sensitization are inconclusive, since in most studies no clear separation is made between pre- and postnatal exposure. However, studies that included prenatal smoke exposure showed no effect on sensitization. Aberrant development of the fetal lung structure, as shown in experimental models, may underlie the increased risk for poor lung function and asthma development. Recently, we showed that maternal smoking during pregnancy decreased expression of genes that are involved in lung development in lungs of neonatal mice. In addition, maternal smoking during pregnancy increased airway remodeling in adult mice offspring. Future experimental studies may reveal whether lung developmental changes may additionally underlie susceptibility to the apparent adult-onset disease chronic obstructive pulmonary disease.

Keywords: cigarette smoke; pregnancy; obstructive lung diseases

Despite the general recognition nowadays of the health risks of maternal smoking on preterm delivery (1), fetal growth restriction (2), and perinatal mortality (3), awareness alone is not sufficient to prompt women to quit smoking. Studies in the United States and in The Netherlands showed a smoking prevalence of respectively 22% and 17% among pregnant women in 2002 (2, 4). In 2008, 38% of smoking women in Amsterdam, The Netherlands, were shown to continue to smoke during pregnancy (5). This number was even higher in the group of women younger than 25 years, in which almost 50% continued smoking (5).

IN UTERO SMOKE EXPOSURE AND DEVELOPMENT OF ASTHMA

Asthma susceptibility has a large genetic component, and it has been firmly established now that lifestyle and interactions between the genetic make-up and environmental exposures, such as parental smoking, determine its development (6). Maternal smoking during pregnancy is an independent risk factor for reduced lung function, as was shown by large studies in North America, several European countries, and Western Australia (7–9). In addition, maternal smoking is a risk factor for physician-diagnosed asthma, wheezing, and respiratory infections in children aged less than 3 years (10, 11), independent of environmental smoke exposure after birth (12), and strongly and independently predicts wheezing after age 16 (13).

Interestingly, a trans-generational effect of smoking during pregnancy on development of asthma was suggested, since grandmaternal smoking during the mother's fetal period was associated with increased asthma risk in her grandchildren (14). This effect was suggested to be due to epigenetic processes, which can be inheritable (15). Growing interest in these processes that can activate or silence genes through alterations in DNA methylation and histone modification resulted in emerging evidence for a role for epigenetic processes *in utero* in increased susceptibility to allergic asthma in offspring (reviewed by Prescott and Clifton [16]). For instance, the severity of ovalbumin-induced allergic airway disease that was inherited transgenerationally was increased when pregnant mice were given high folate diets (i.e., a source of methyl donors) (17). This was accompanied by hypermethylation (i.e., suppression) of the *Runx3* gene in the offspring. *Runx3* cooperates with T-bet in the silencing of IL-4 in the Th1 cell (18), contributing to the observed severity of disease. Interestingly, a very recent study by Breton and coworkers showed that maternal smoking during pregnancy was associated with global hypomethylation and CpG-specific DNA hypermethylation of eight genes (19) in children. This is of interest, since global hypomethylation is believed to result in chromosomal instability and increased mutation events, whereas promoter hypermethylation can affect gene expression.

In all mentioned studies a clear separation was made between the effects of maternal smoking during pregnancy and postnatal cigarette smoke exposure to determine independently the effect of intrauterine exposure. Whether intrauterine exposure affects development of atopic asthma or nonatopic asthma is difficult to distinguish from the studies that report asthma and wheezing in children less than 6 years of age. This makes it hard to speculate on the underlying mechanisms. However, since maternal smoking during pregnancy increased the risk for asthma in smoking nonatopic adolescents (20), it could very well be that the excess incidence of wheezing in smoking households is largely attributable to nonatopic "wheezy bronchitis," as suggested by Strachan and Cook (21). Here it was proposed that parental smoking is a co-factor, rather than an underlying cause, provoking wheezing attacks and more severe disease among children with established asthma.

We recently found that maternal smoking during pregnancy (and not postnatal) increased responsiveness to methacholine, smooth muscle layer thickness, and collagen III deposition around the airways in mouse offspring, irrespective of house dust mite (HDM) exposure. Maternal smoking during pregnancy had no effect on (HDM-induced) allergic inflammation, such as numbers of eosinophils or expression of the Th2 cytokines IL-4 and IL-5 in offspring (22). This suggests that the observed changes in the airways may underlie susceptibility

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to development of nonatopic asthma, rather than atopic asthma or sensitization, but may also increase susceptibility to chronic obstructive pulmonary disease (COPD).

IN UTERO SMOKE EXPOSURE AND IMMUNOGLOBULIN E SENSITIZATION

Development of allergies is also largely determined by a genetic predisposition (23), but data on the association between exposure to tobacco smoke in childhood and risk of atopic sensitization remain inconclusive, since most studies lack a clear separation between prenatal and postnatal smoke exposure. However, when *in utero* smoke exposure was included, little or no association with sensitization to inhalant allergens was found, as shown in studies from Germany, the United Kingdom, Italy, and the Netherlands (24–28). This confirms data from a prospective birth cohort study (BAMSE) from Sweden in which a clear separation between prenatal and postnatal smoke exposure was made and no consistent effect on sensitization was seen among children exposed to smoke only *in utero* (11). However, in contrast, postnatal exposure in early infancy increased the risk of sensitization to indoor inhalant and food allergens (11). Interestingly, a recent study in 1,314 German children showed that regular maternal smoking is a strong risk factor for allergic sensitization and asthma symptoms during the first 10 years of life, but only in children with allergic parents (29). In this study no clear separation was made between prenatal and postnatal exposure as well, but it indicates that gene–environment interactions play a role in allergic sensitization.

In our mouse model for HDM-induced asthma, we additionally found no effects of maternal smoking during pregnancy on HDM-induced sensitization in offspring for both total and HDM-specific immunoglobulin (Ig) E (22).

IN UTERO SMOKE EXPOSURE AND DEVELOPMENT OF COPD

COPD is conventionally thought of as a disease of adult smokers. However, early origin for COPD is supported by observations that childhood lower respiratory tract illness, possibly caused by early viral or bacterial infections or low birth weight, is associated with airflow limitation in adulthood (30–32). Maternal smoking may contribute to adult airflow limitation, since children exposed to maternal smoking are at risk for childhood respiratory infections and low birth weight (33). Recently, maternal smoking was shown to be associated with lower lung volume irrespective of personal smoking (34). Furthermore, in that same study it appeared that maternal smoking synergized with personal smoking to increase airflow limitation and COPD.

No animal studies are available on *in utero* smoke exposure and susceptibility for development of COPD. However, in an animal study in which neonates were exposed to cigarette smoke for 2 weeks, the type 1 and type 2 interferon (IFN) pathway gene expression was inhibited and oxidative stress, alveolar cell death, and transforming growth factor-β signaling were increased in neonatal lungs. This was followed by impaired alveolar growth in adult lungs (35). Down-regulation of the IFN pathway gene expression may underlie enhanced susceptibility to respiratory infections (36). This is supported by a study from Phaybouth and colleagues showing that smoke-exposed mouse neonates had a diminished immune response to respiratory syncytial virus infection (37). Both experimental studies indicate that the postnatal period is particularly susceptible to the detrimental effects of cigarette smoke, and these studies are relevant to infants born of mothers that smoked during and after pregnancy or infants exposed to secondhand smoke both during pregnancy and after delivery.

Another reason to refrain from smoking during pregnancy is that maternal smoking was found to synergize with personal smoking to increase airflow limitation and risk for development of COPD (34). To further investigate the risk of maternal smoking on development of COPD, longitudinal studies (in mice and in humans) are needed, since no cohort study has been running long enough to follow patients from early childhood until senescence.

MECHANISMS INVOLVED

The mechanisms underlying the increased risk for development of asthma in infants exposed to maternal tobacco smoking during pregnancy are largely unknown. Several *in vitro* and experimental animal studies have shown that maternal smoking during pregnancy affects the neonatal immune system development, lung structure, and lung function in offspring (38–40).

With respect to lung structure, maternal smoking during pregnancy increased the size and decreased the number of alveoli in rat offspring (38), while maternal nicotine exposure during pregnancy increased collagen deposition around large airways and vessels in rhesus monkey offspring (39). Furthermore, in children who died from sudden infant death syndrome, evidence for structural changes in lung tissue due to maternal smoking was present as well, since the children from smoking mothers had a higher inner airway wall thickness than did children from nonsmoking mothers (41). The mechanisms causing these changes in lung structure are not entirely clear yet, but literature points toward a role for nicotine in decreasing overall glycolysis in lung structural cells and increasing apoptosis (reviewed by Maritz [42]). In addition, a role for nicotine in increasing the number and metabolism of alveolar type II cells (43, 44), which are the cells synthesizing surfactant in the developing lung, was proposed. Via yet unknown mechanisms, these processes may cause changes in the normal developmental pattern of the *in utero* exposed lung.

Recently, we found lower expression of genes involved in the Wnt signaling pathway in newborn (Day 1) mouse offspring from mothers exposed to cigarette smoke during pregnancy (45). Wnt signaling plays an important role in lung development (46). For instance, conditional inactivation of the β-*catenin* gene in the epithelium of the developing mouse lung leads to neonatal death resulting from severe lung defects (46). Interestingly, the β-*catenin* gene was one of the genes that were down-regulated in our study. Consequences for protein expression and alveolarization are currently under investigation, as is the question whether epigenetic mechanisms underlie this decrease in gene expression.

SUMMARY

The effects of maternal smoking during pregnancy on sensitization, lung function, and asthma development have been extensively studied during recent years, and most epidemiological studies show an association with poor lung function and wheezing in early childhood. However, epidemiological data with respect to sensitization and development of atopic asthma are inconclusive albeit that maternal smoking during pregnancy has been associated with *severity* of asthma. With respect to effects of *in utero* smoke exposure and development of COPD, longitudinal studies are needed to confirm the involvement of maternal smoking during pregnancy, and to establish the underlying biological mechanisms.

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